Anadolu Üniversitesi Bilim ve Teknoloji Dergisi C- Yaşam Bilimleri ve Biyoteknoloji
Anadolu University Journal of Science and Technology C- Life Sciences and Biotechnology

Year: 2018
Volume: 7
Number: 2
Page 220 - 226
DOI: 10.18036/aubtdc.364844

HISTOLOGICAL EXAMINATION OF THE ROLE OF VITAMIN E IN CYCLOPHOSPHAMIDE-INDUCED TESTICULAR DAMAGE IN RATS

Dilek BURUKOĞLU DÖNMEZ [1, *], İnci YETİM [1]

[1] Department of Histology and Embryology, Medical Faculty, Eskişehir Osmangazi University, Eskişehir Türkiye

ABSTRACT

Cyclophosphamide is among anticancerous and immunosuppressing drugs frequently used in chemotherapy treatment. Cyclophosphamide is a chemotherapeutic agent but has also toxic effect on testes. Testicular cells can be protected from toxic effects by antioxidant systems, including vitamin E. The aim of this study is to reveal what kind of effects the vit-E exerts upon experimentally induced CP toxicity in testicular tissue of rats. Results of the present study indicate that the testicular injury induced by CP can be prevented by administration of vitamin E.

Keywords: Cyclophosphamide, Vitamin E, Rat, Testis

SIÇANLARDA SİKLOFOSFAMİDE BAĞLI TESTİS HASARINDA VİTAMİN E'NİN ROLÜNÜN HİSTOLOJİK OLARAK İNCELENMESİ

ÖZET

Siklofosfamid, kemoterapi tedavisinde sıklıkla kullanılan antikanser ve immünosüpresan ilaçlar arasındadır. Siklofosfamid kemoterapötik bir ajandır ancak testis üzerinde toksik etkiye sahiptir. Testis hücreleri, E vitamini gibi antioksidan sistemler ile bu toksik etkilerden korunabilir. Bu çalışmanın amacı, vit E'nin sıçanlarda testiküler doku üzerinde deneysel olarak uyarılan CP toksisitesine ne tür bir etki uyguladığını açığa çıkarmaktır. Bu çalışmanın sonuçları, CP ile indüklenen testikular hasarın vitamin E'nin verilmesiyle önlenebildiğini göstermektedir.

Anahtar Kelimeler: Siklofosfamid, E vitamini, Sıçan, Testis

1. INTRODUCTION

Cancer is a fatal disease that demonstrates itself by uncontrolled reproduction of normal cells, their proliferation and causing metastasis. In addition to being a treatment that halts the growth and reproduction of tumor cells or completely destroys them, antineoplastic chemotherapy could also damage normal cells due to the fact that normal cells and tumor cells do not greatly differ in terms of their structures [1, 2]. It is commonly known that, as a result of the use of antineoplastic drugs in chemotherapy, significant toxic effects are produced on gastrointestinal system, hematopoietic system cells, and testicles due to normal cell destruction [2]. Cyclophosphamide (CP) is among anticancer and immunosuppressing drugs frequently used in chemotherapy treatment. As an alkylating agent, CP should be administered in high doses. However, the use of this approach is restricted due to such side effects as hematotoxicity, urea toxicity, and hepatotoxicty. The most significant side effect of CP is reproductive toxicity in animals and humans. Spermatogenic cells are quite susceptible to detrimental effects of chemotherapeutic drugs. These drugs may lead to permanent infertility by causing irreparable damage in stem cell colony. The most susceptible cells are actively dividing spermatogonia and spermatocytes up to preleptotene phase [3]. In adult male patients, spermatogenesis stops in testicular tissue and sperm count reduces as a result of treatment with CP [1]. The long-term use of CP during treatment causes a decrease in fertility and in the weight of reproductive organs [4]. Vitamin E (vit-E)

*Corresponding Author: dburukoglu@yahoo.com
Receiving Date: 12 December 2017 Publishing Date: 17 August 2018

is comprised of 8 tocopherols and is soluble in fat. The most commonly found and active one among these 8 tocopherols is alpha-tocopherol. Vit-E is an important antioxidant for biological systems and it is found in high concentrations in tissues, especially in sections rich in membrane [5]. In these membranous parts, it prevents cell damage by breaking lipid peroxidation and, in addition, intracellular signaling in normal manner is possible with the presence of this vitamin [5, 6]. Free radicals appear during the normal activity of oxidative enzymes and univalent reduction of molecular oxygen. Radicals not separate by themselves with cause inactivation of sperms as a result of the peroxidation of phospholipids in sperm mitochondria [7, 8]. Vit-E, however, prevents this effect of free radicals from happening. The aim of this study is to reveal what kind of effect the vit-E exerts upon the experimentally induced CP toxicity in testicular tissue of rats.

2. MATERIALS AND METHODS

A total of 32, 4 months old adult male Sprague Dawley rats with weights ranging 250-300g were used in our study. All the procedures performed in this study were approved by Eskişehir Osmangazi University Animal Experiments Local Ethics Committee by decision no 185-1 dated 02.02.2011. Experimental animals were divided into 4 groups. Control group: Rats belonging to control group were intraperitoneally given normal saline (since it dissolves CP) and olive oil (since it dissolves vit-E) at a dose determined by estimating the volume according to body weights of once a day for 7 days. CP group: This group was given CP dissolved in normal saline in 20mg/kg once a day for 7 days. Then, olive oil was administered also intraperitoneally by estimating the volume according to body weights of the animals. CP + vit-E group: This group was given 20mg/kg CP once a day for 7 days and then 200mg/kg vit-E was administered intraperitoneally immediately after the application [9]. Vit-E group: This group was given 200mg/kg vit-E once a day for 7 days and then normal saline was administered intraperitoneally at a determined dose immediately after the application. Right from the beginning of the experiment, body weights of rats were weighed daily in order to determine the doses of substances to be administered. After the body weights were weighed and recorded at the end of the 7^{th} day, rats were then anesthetized with intraperitoneally administered ketamine (ketalar 90 mg/kg) + xyilazin (alfazyne 10 mg/kg) and their abdomens were opened and testes were removed. Each of the testes completely cleared from the peripheral tissues was weighed by Ohaus (Adventurer Pro AV264C) brand precision scale. Testes were then immersed in Bouin's solution and kept for 24 hours. Then, paraffin blocks were prepared following routine tissue follow-up procedures. Serial sections of 3 μm thickness were cut from the paraffin blocks and the sections were then stained with PAS+H, H-E, and Toluidin blue for microscopic examination. For histological assessment, the stained sections were examined under the Olympus BX51 light microscope (Olympus Corp. Tokyo, Japan) equipped with DP 70 digital camera and images that represented the groups were then obtained.

3. FINDINGS

Control group: Testicular basal lamina structure, seminiferous tubular structures and all the cells it contains, interstitial region and Leydig cells found in that region, venous structures, and mast cells were observed to have normal histological structures (Figure 1a-d).

CP group: Severe damage in seminiferous tubules, severe degeneration and cellular losses in spermatogenic cells along with thin tubule wall were observed. In addition, basal lamina was found to have been separated from tubule wall. None of any phases of spermatogenic cells and spermatogenesis were distinguishable in tubule wall. Also, edema and venous congestion were established in interstitial region. Cellular desquamation, tubular necrosis, and thickening in basal lamina were observed as well. Macrophage and Leydig cells found in interstitial region were established to be in normal structure; and mast cells were found to have increased in number (Figure 2a-d).

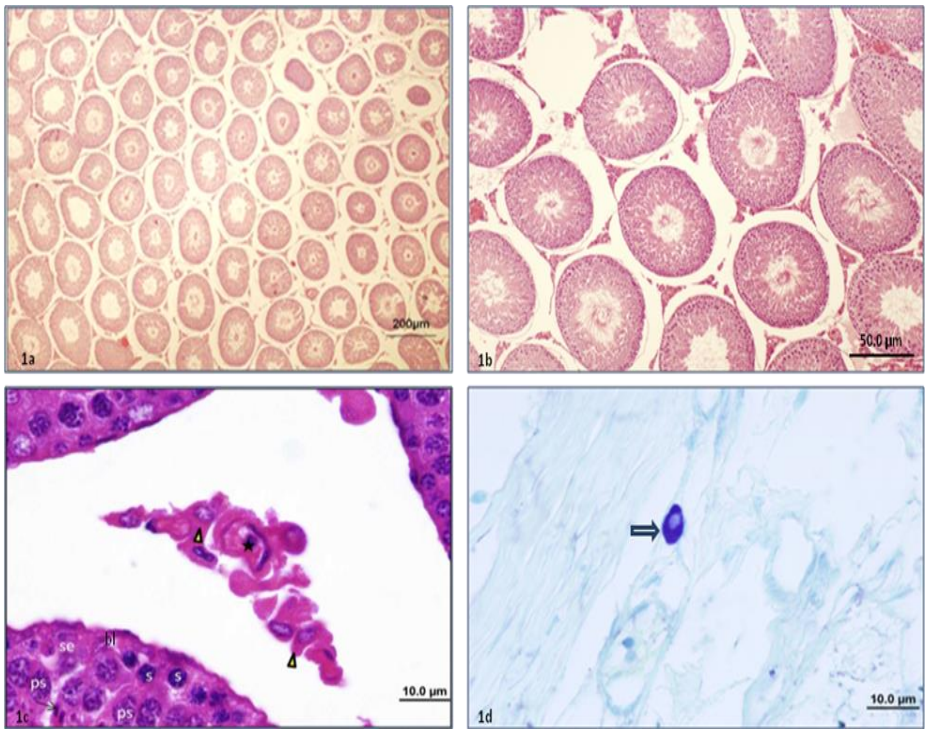

Figure 1: Control group: Testes seminiferous tubule structures with normal appearance, spermatogenic cells, Sertoli cell (Se), spermatogonium (s), primary spermatocyte (ps), interstitial region (a-c) and the structures, Leydig cell (►), venous structure (*) (c) and mast cells (arrow) (d) are observed here.(HE, PAS+H, Toluidine blue), (bar:200µm, bar:50.0µm, bar:10.0µm).

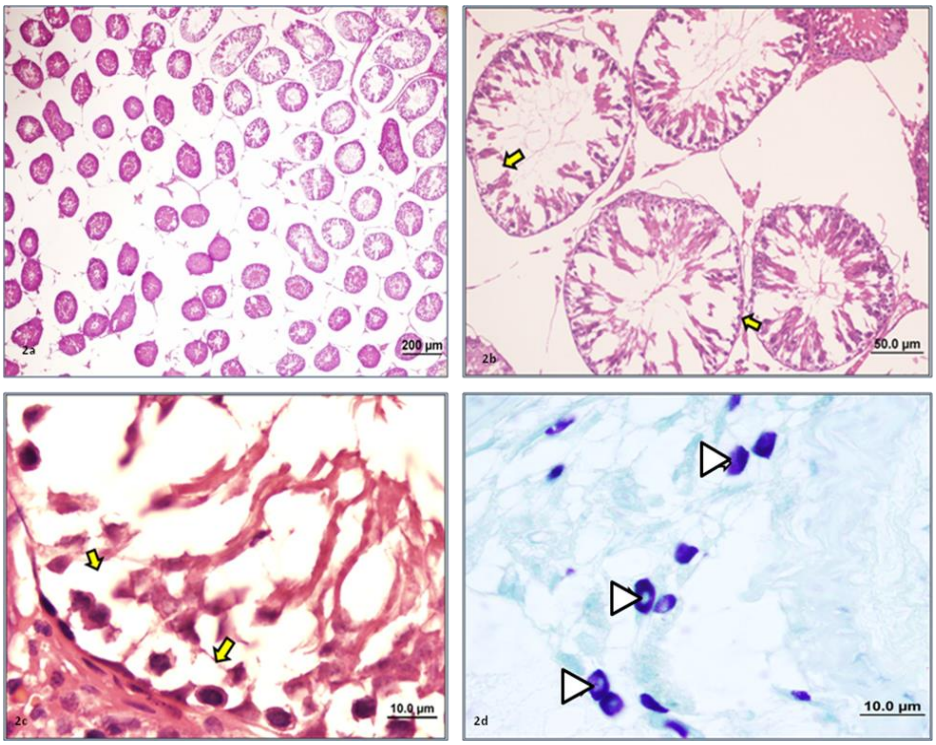

Figure 2: Cyclophosphamide group: Severe damage in seminiferous tubules, degenerations and cellular losses in spermatogenic cells in seminiferous tubules along with thin tubule wall were observed (→) (a-c). Mast cells (►) found in interstitial region stand out to have been increased in number (d). (HE, PAS+H, Toluidine blue), (bar:200µm, bar:50.0µm, bar:10.0µm).

Vit-E group: Seminiferous tubule structures and spermatogenic cell series demonstrated a structure similar to control group. Spermatogenesis was observed to follow its normal course. Interstitial region and Leydig cells, venous structures, and mast cells were established to be normal (Figure 3a-d).

CP+vit-E group: Although it was observed that damage was maintained in few tubules, though less, decreased tubular damage, maintained spermatogenic cells and spermatogenesis was established to continue. Cellular defects found in spermatogonium and occasionally primary spermatocytes in groups that were only given CP were rarely observed in groups that were administered CP+vit-E. Besides, PAS positive stained basal lamina structure around the tubule was observed to be normal. Leydig cells with near normal appearance and mast cells stained with Toluidin blue were established in interstitial region (Figure 4a-d).

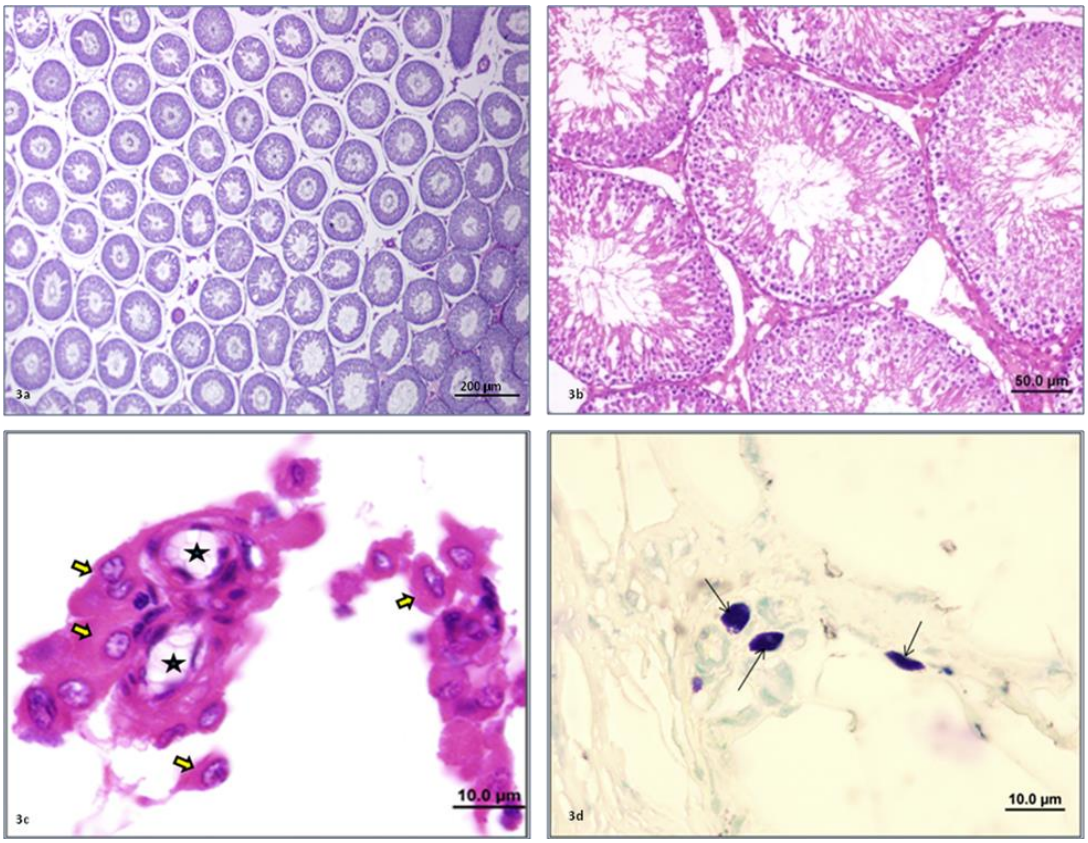

Figure 3: Vitamin E group: Seminiferous tubule structures with near normal appearance, spermatogenic cells in tubule wall (a,b), interstitial region and structures, Leydig cell (thick arrow), venous structure (*) (c) and mast cells (thin arrow) are observed (d). (HE, PAS+H,Toluidine blue), (bar:200µm, bar:50.0µm, bar:10.0µm).

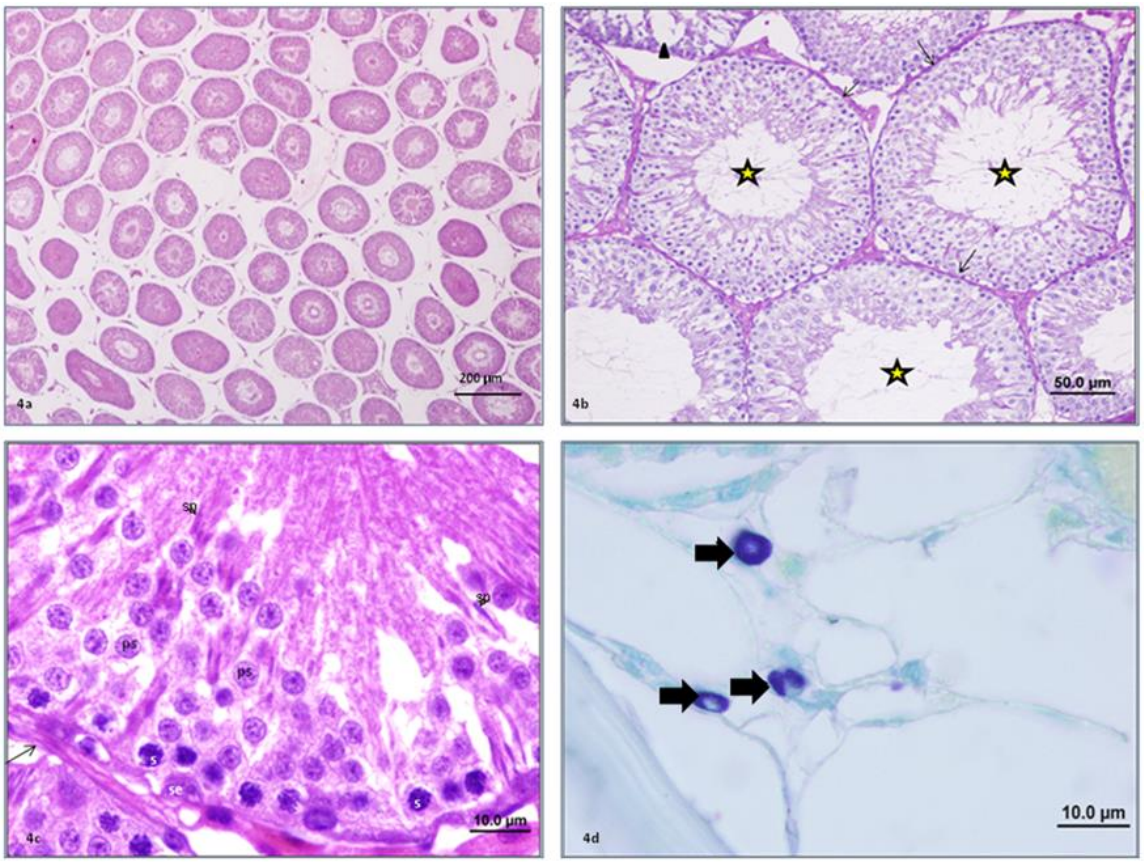

Figure 4: CP+Vitamin E group: Although it is observed that damage is maintained in few tubules (►), though less, decreased tubular damage, maintained spermatogenic cells (*), Sertoli cell (Se), spermatogonium (s), primary spermatocyte (ps), spermatid (sp), and spermatogenesis is observed to continue (a-c). Basal lamina structure around the tubule is observed to be normal (→) (c). Mast cells (thick arrow) with a near normal count are found in interstitial region (d). (HE, PAS+H, Toluidine blue), (bar:200µm, bar:50.0µm, bar:10.0µm).

4. RESULTS AND DISCUSSION

CP is an effective chemotherapeutic agent. While drugs that are used in cancer chemotherapy destroy cancer cells, they can negatively affect normal tissues as well. It was also demonstrated by some studies that CP caused renal disorders during chemotherapy treatment, in some cancer types, infertility in adult males and children when used alone or in combination, decrease in testicular functions and reduction in the weight of reproductive organs [4, 10]. In their study, Rezvanfar M.A. et al. found that CP given to rats every day for 28 days as i.p 6mg/kg negatively affected spermatogenesis, decreased sperm quality and severely damaged seminiferous tubules. Dispersion and hypoplasia in Leydig cells, interstitial edema, and also decrease in sperm count and activity were also demonstrated [11]. In this study too, severe damage in seminiferous tubules and edema in interstitial region were observed. However, any damage to Leydig cells was not established. In a study by Sabik and Abd el-Rahman conducted on rats, it was reported that spermatogenic cells in seminiferous tubules were degenerated, dispersion, cellular degeneration, and necrosis were observed in spermatogonium cell layer along with atrophic changes in seminiferous tubules, spermatogenesis was prevented, and that giant cells containing too many nuclei in seminiferous tubules were found in groups that were given 20mg/kg CP every day for 7 days. It was also reported in the same study that spermatogenesis went back to normal and cellular damage was reduced in the group that were administered vit-E along with CP [12]. Hoda H. et al. assessed toxic effects of cyclophosphamide for 4 weeks on rat testis histologically and immunohistochemically. No preservative was used. The authors reported that chronic administration of cyclophosphamide treatment

elicited serious histological changes in testis, which was maintained after discontinuance of the drug. In our study, we also investigated toxic effects of the cyclophosphamide on testes. However, duration of our experiment was seven days, and vitamin E was administered as preservative beside cyclophosphamide [13]. Aml F. Elgazar evaluated toxic effects of cyclophosphamide on testes by light microscopy during the 6-week period of experiment. Walnut extract and vitamin E was used to prevent or reduce toxic effects. The study reported antioxidant activity by combined administration of walnut extract and vitamin E. Our study exhibited similar characteristics to this study, except the seven-day duration of our experiment and no use of vitamin E as preservative in addition to cyclophosphamide. While only hematoxylin eosin was used as a histological dye for evaluation under light microscopy in this study, we used various staining methods such as hematoxylin eosin, PAS+HE, toluidine blue to evaluate different kinds of cells [14]. Mohammad Afkhami-Ardakani et al. assessed toxic effects of cyclophosphamide on testes by light microscopy during the 4-week period of experiment. Spirulina platensis (Arthrospira platensis) was used to prevent or reduce toxic effects. The authors reported a stronger antioxidant activity with Spirulina platensis (Arthrospira platensis) than that with vitamin E. Our study exhibited similar characteristics to this study, except the seven-day duration of our experiment and no use of vitamin E as preservative apart from cyclophosphamide. While only hematoxylin eosin was used as a histological dye for evaluation under light microscopy in this study, we used various staining methods such as hematoxylin eosin, PAS+HE, toluidine blue to evaluate different kinds of cells. Toluidine blue helped to also visualize mast cells in detail [15]. It was observed that CP used in doses in this study as well caused atrophy in testicular seminiferous tubules and damage cell series, and that vit-E used in combination with CP reduced the said damage.

Considering the duration and dose administered in this study, CP was established to be quite toxic for testes and to have impaired histological structure. CP was also observed to negatively affect spermatogenesis by causing severe damage to spermatogenic cells and cellular desquamation, and to lead to thickening in basal lamina, severe damage in tubules, and atrophy. In addition, CP caused a significant decrease in body weight and total testicular weight. Vit-E that has an antioxidant effect was found to have significantly decreased the damage caused by CP to testes. Advanced enzymatic, biochemical, and immunohistochemical studies on revealing how vit-E prevents CP toxicity may provide additional information and evidence on the issue.

5.REFERENCES

[1] Howell S, Shalet S. Gonadal damage from chemotherapy and radiotherapy. Endocrinol. Metab. Clin. North. Am 1998; 27: 927–4.3

[2] Kayaalp O. Vitaminler, E vitamini, Rasyonel tedavi yönünden tıbbi farmakoloji. 1.baskı. Ankara Türkiye: Hacettepe-Taş Kitapçılık, 2009.

[3] Meistrich ML, Wilson G, Kangasniemi M. Mechanism of protection of rat spermatogenesis by hormonal pretreatment: stimulation of spermatogonyum l differentiation after irradiation. Journal of Andrology 2000; Vol 2.

[4] Papageorgio C, McLeod HL Chemoterapy: Principles and Pharmacology. The Washington Manual of Oncology Philadelphia, Lippincott, Williams-Wilkins, 2002.

[5] Estakhri R, Hajipour B, Majidi H, Soleimani H. Vitamin E ameliorates cyclophosphamide induced nephrotoxicity. Life Science Journal 2013;10:6.

[6] Nagel E, zu Vilsendorf AM, Bartels M, Pichlmayr R. Antioxidative vitamins in prevention of ischemia/reperfusion injury. Internat J Vit Nutr Res 1997; 67: 298-306.

[7] Lamirande E, Gagnon C. Reactive oxygen species and human spermatozoa II. Depletion of adenosine triphosphate plays an important role in the inhibition of sperm motility. J Androl 1992; 13: 379-386.

[8] Palamanda JR, Kehrer JR. Involvement of vitamin E and protein thiols in the inhibition of microsomal lipid peroxidation by glutathione. Lipids 1993; 28:427-431.

[9] Sarkar SD, Maiti R, Ghosh D. Management of fluoride induced testicular disorders by calcium and Vitamin-E co-administration in the albino rat, Reproductive Toxicology 2006; 22: 606-612.

[10] Chapman RM. Gonadal injury resulting from chemotherapy. Am J Indust Med 1983; 4:149-161.

[11] Rezvanfar MA, Sadrkhanlou RA, Ahmadi A, Shojaei-Sadee H. Protection of cyclophosphamide-induced toxicity in reproductive tract histology, sperm characteristics, and DNA damage by an herbal source; evidence for role of free-radical toxic stres. Hum Exp Toxicol 2008; 27, 901.

[12] Laila ME. Sabik, Sahar S.Abd.El-Rahman. Alpha-tocopherol and ginger are protective on cyclophosphamide-induced gonadal toxicity in adult male albino rats. Basic and Applied Pathology 2009; 2: 21-29

[13] Hoda H Anan, Nashwa S Wahba, Maha A Abdallah, Dalia A Mohamed. Histological and immunohistochemical study of cyclophosphamide effect on adult rat testis. International Journal of Scientific Reports 2017; 3, 39-48.

[14] Aml F, Elgazar. Protective role of walnut seeds extract and vitamin E against testicular toxicity induced by cyclophosphamide in male rats. British Journal of Medicine & Medical Research 2016; 18: 1-10.

[15] Mohammad Afkhami-Ardakani, Shapour Hasanzadeh, Rasoul Shahrooz, Norouz Delirezh, Hassan Malekinejad. Antioxidant effects of Spirulina platensis (Arthrospira platensis) on cyclophosphamide-induced testicular injury in rats. Veterinary Research Forum 2018; 9: 35 – 41.